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PARTIAL INHIBITION OF IN VITRO POLLEN GERMINATION BY SIMULATED SOLAR ULTRAVIOLET-B RADIATION[1]

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Abstract. Pollen from four temperate-latitude taxa were treated with UV radiation in a portion of the UV-B (280–320 nm) waveband during in vitro germination. Inhibition of germination was noted in this pollen compared to samples treated identically except for the exclusion of the UV-B portion of the spectrum. Levels similar to maximum solar UV-B found in temperate-latitude areas failed to inhibit pollen germination significantly, while levels similar to maximum solar UV-B found in equatorial alpine locations caused partial inhibition of germination in three of the four taxa examined.

Key words: *ozone reduction; pollen germination; radiation; solar ultraviolet; ultraviolet-B.*

INTRODUCTION

Pollen may be damaged by ultraviolet radiation of wavelengths that occur in solar radiation. Although pollen appears to be well shielded from solar UV when still within the anther sacs (Flint and Caldwell 1983), it may be exposed to high insolation following dehiscence until successful germination and stigma penetration occur. Potential damage to pollen by solar UV radiation has received little attention even though UV damage to vegetative organs has been investigated frequently in the last decade (Caldwell 1981, Teramura 1983). Suggestions that a reduction of the atmospheric ozone layer may occur, resulting in enhancement of UV radiation and a shift to shorter wavelengths within the UV-B (280–320 nm) waveband (National Academy of Sciences (USA) 1982) provide further impetus to assess the potential susceptibility of pollen to UV-B radiation.

The study of Werfft (1951) has often been cited as evidence that solar UV injures plant pollen (Linskens 1964, Proctor and Yeo 1972, Stanley and Linskens 1974). In her study, dry pollen was placed in UV-B transparent cellophane envelopes and was either exposed to sunlight or shaded. Although decreased germinability was observed in the sunlight treatment, a temperature increase due to solar heating or other factors could have been responsible, and thus this study is inconclusive.

Radiation of very short wavelength (UV-C, <280 nm) can damage pollen to the extent of reduced viable seed-set and induction of mutations (Stadler and Uber 1942, Steinitz-Sears and Sears 1957, Pfahler and Linskens 1977*a*, *b*) or decreased in vitro germination (Werfft 1951, Brewbaker et al. 1965). However, there is no UV-C radiation in the solar spectrum at the Earth's surface, and even with a massive 90% reduction of the ozone layer, there would still be no UV-C radiation (Caldwell 1981). Reliable procedures for estimating solar UV-B damage by extrapolating from studies using UV-C irradiation (254-nm) do not appear feasible (Nachtwey 1975), precluding use of these studies in estimating pollen response to solar ultraviolet.

Recently, attempts have been made to examine the effects of polychromatic UV-B radiation (using a spectral composition resembling sunlight) on pollen germination. Pfahler (1981) found a slight but statistically significant inhibition of germination in maize pollen treated during in vitro germination with very high levels of UV-B from a filtered fluorescent sunlamp. Chang and Campbell (1976), using lower levels of irradiation, found inhibition of in vitro pollen germination in two clones of *Tradescantia* after 1.5 h of treatment. However, these clones are interspecific hybrids not found in nature; thus, their sensitivity may not be representative of most angiosperm taxa. To determine whether pollen is likely to be damaged by either current or enhanced levels of solar UV-B, we treated germinating pollen from several species with UV-B from filtered fluorescent sunlamps and assessed in vitro inhibition of germination.

METHODS

Pollen grains were germinated on a solid medium containing sucrose, 0.6% agar, 0.03% $CaNO_3$, and 0.01% boric acid (Pfahler 1981). Using a 15% sucrose medium, average control germination was 47% in *Papaver rhoeas*, 57% in *Cleome lutea*, and 60% in *Scrophularia peregrina*. A 34% sucrose medium was required for germination of *Geranium viscosissimum* var. *nervosum* pollen, but an average control germination of only 18% was achieved. As taxa in the Geraniaceae have trinucleate pollen (Brewbaker 1967), which is often difficult to germinate in vitro, this low germination

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percentage was not unexpected. This medium was applied to microscope slides and placed on moist filter paper in shallow Petri plates. A plastic film secured over the dish prevented loss of water vapor and also selectively filtered the UV radiation in the controlled environment chamber. Fluorescent sunlamps (Westinghouse FS-20), whose output is primarily UV-B and short-wavelength UV-A, were the only source of irradiation. Samples being irradiated with UV-B were covered with clear cellulose acetate film (0.13 mm thick) which transmits UV-B radiation but absorbs UV-C. As a control, DuPont Mylar-D was used, as this film absorbs UV-B and UV-C but otherwise has transmittance properties similar to cellulose acetate. Sisson and Caldwell (1975) describe irradiance from this lamp/filter system. Each percent inhibition value in Fig. 1 represents a separate experiment conducted on a different day. The irradiance was varied by changing the distance between the lamps and the Petri plates for different experiments.

Pollen collected from greenhouse-grown plants was sprinkled onto agar-covered slides, immediately placed into filter-covered Petri plates and then into the 25°C irradiation chamber. Plates (three to five replicates per experiment) were assembled alternating sequentially between the treatment and control groups. Germination was terminated at the end of the irradiation period (3 h except where noted) with a killing and preserving solution (Pfahler 1981). Percent germination was analyzed with a paired t test (after using the arcsin [proportion]$^{-2}$ transformation).

Ultraviolet irradiance was measured with either a modified Gamma Corporation (San Diego, California) high-resolution grating spectroradiometer (model 2900 photometer and model 700-31 monochromator) or a double-grating Optronic Corporation (Orlando, Florida) model 742 monochromator. Although the spectral distribution of cellulose-acetate-filtered FS-20 sunlamps approximates the solar UV-B spectrum, the pronounced wavelength dependence of UV-B damage in biological systems precludes a simple comparison of total UV-B flux between the experimental doses and ambient levels of solar UV-B. However, by multiplying spectral irradiance at each wavelength by a weighting function, a comparison can readily be made. As UV-B radiation damages DNA in pollen (Jackson and Linskens 1979), we utilized a weighting function describing UV damage to DNA (Setlow 1974). This weighted irradiance, when integrated between 285 and 320 nm, is termed DNA-damaging irradiance and is expressed in units of effective milliwatts per square metre.

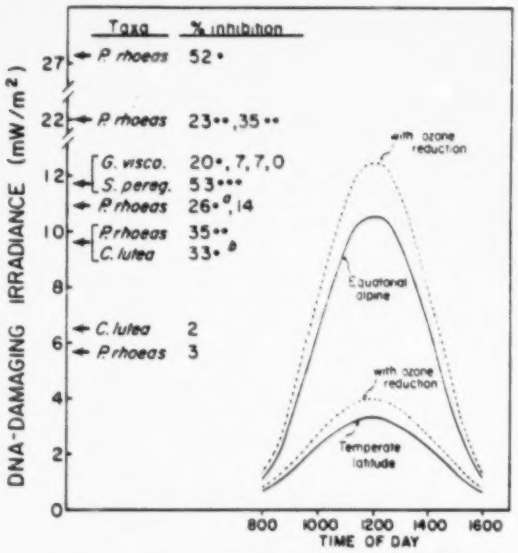

FIG. 1. Percentage inhibition of pollen germination by different levels of simulated solar UV-B irradiation. Each percent inhibition value represents a separate experiment conducted on a different day. Taxa are *Papaver rhoeas* cv. Shirley Single (Papaveraceae), *Geranium viscosissimum* var. *nervosum* (Geraniaceae), *Cleome lutea* (Capparidaceae), and *Scrophularia peregrina* (Scrophulariaceae). Arrows to the ordinate indicate irradiance employed in different experiments; irradiation period was 3 h except where indicated by superscript letters: [a] 2-h irradiation followed by 1 h darkness; [b] 3.3-h irradiation. Significance levels: $*P < .10$, $**P < .05$, $***P < .025$. Values not followed by a symbol did not differ statistically from controls. For comparison, current levels of solar UV are represented by solid lines and are taken from earlier clear-sky measurements (Caldwell et al. 1980); UV following 7% ozone depletion is represented by dashed lines and calculated using the model of Green et al. (1980). All radiation measurements were weighted with the DNA-damage function of Setlow (1974).

Results and Discussion

Partial inhibition of pollen germination for the four species is depicted in relation to different irradiance levels in Fig. 1. The diurnal course of DNA-effective irradiance is also shown in Fig. 1 for midlatitude (Logan, Utah, USA) and equatorial alpine (Peru and Venezuela) locations at the time of year of maximum solar radiation from earlier measurements (Caldwell et al. 1980). Irradiance for a 7% ozone depletion, which is the current predicted equilibrium value (National Academy of Sciences 1982), was calculated using the model of Green et al. (1980). Ultraviolet irradiance on the order of that currently received during midday at equatorial alpine locations was generally effective in causing partial inhibition of pollen germination except in *G. viscosissimum*. However, even at the highest irradiance employed, inhibition of pollen germination was not complete.

The four species we examined were of temperate-latitude origin. These species are insect pollinated, and except for *S. peregrina*, the reproductive organs are fully exposed to direct sunlight. Pollen germination of these species showed no significant inhibition at UV

irradiance levels similar to those occurring at midlatitudes. Only when irradiance similar to that found at low latitudes was used did inhibition of germination become significant.

Scrophularia peregrina was the most sensitive species examined, perhaps because its tubular corolla may provide partial shielding of pollen from direct solar UV. Corolla tissue generally shows very low UV-B transmittance (Flint and Caldwell 1983). However, the open end of the corolla could still admit substantial levels of diffuse UV-B. *Geranium viscosissimum* showed the least inhibition. This was the only trinucleate pollen species examined. Maize pollen, which similarly showed only slight inhibition under UV-B irradiation (Pfahler 1981) is also shed in the trinucleate condition (Brewbaker 1967). At the time of anther dehiscence, maturation of metabolic features in trinucleate pollen is more advanced than in binucleate pollen (Hoekstra and Bruinsma 1979). This trinucleate condition may provide more resistance to UV-B damage. An alternative explanation could be that differences in sporoderm thickness and structure could determine pollen sensitivity to UV.

Applying our results to temperate-latitude taxa involves several assumptions. The DNA-damage irradiance weighting function must be appropriate, the species must be representative of temperate-latitude taxa, and in vitro experiments must adequately simulate in vivo field conditions. If these assumptions are correct, many taxa native to temperate latitudes should not experience inhibition of pollen germination even after ozone depletion. The sensitivity of pollen from species native to the tropical highlands remains unknown, as does the possibility of synergistic effects between anthropogenic pollutants (e.g., SO_2 [Varshney and Varshney 1981]), natural stress factors (e.g., low humidity [Henny 1980]), and ultraviolet radiation.

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